A New 16-Membered Chalcomycin Type Macrolide Antibiotic, 250-144C

YANG MO GOO, YOUN YOUNG LEE† and BUM TAE KIM†

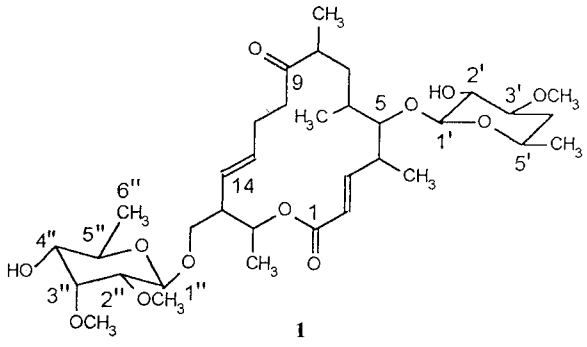
Department of Pharmacy, †Department of Chemistry, Seoul National University, Seoul 151-742, Korea

(Received for publication June 17, 1996)

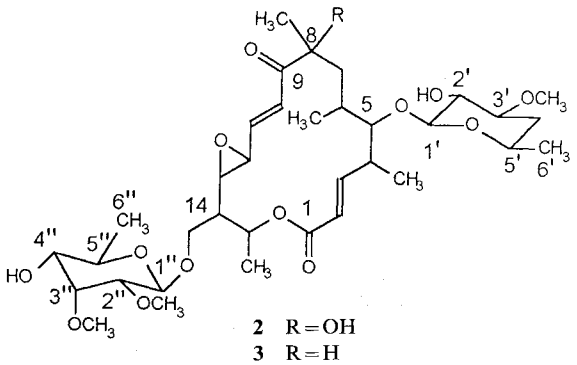
Since the discovery of carbomycin[1] in 1952 more than seventy 16-membered macrolide antibiotics have been isolated and characterized[2]. The macrolides consist of an aglycon skeleton to which basic amino or neutral sugars are attached. Three different carbon skeletons, which show characteristic ultraviolet absorption spectra have been characterized. They are represented by leucomycin, tylosine and aldgamycin. So far, five different types of chromophores, $\alpha,\beta,\gamma,\delta$-unsaturated alcohol (232 nm, strong), epoxide-α,β-unsaturated ketone (240 nm, strong), $\alpha,\beta,\gamma,\delta$-unsaturated ketone (280 nm strong), epoxide-allylic alcohol (end), and α,β-unsaturated ketone with α,β-unsaturated lactone (240, strong and 216 nm, medium) are found in the aglycone part[3].

During the course of screening of new antibiotics from microbial source, we isolated *Streptomyces* sp. SNUS-9011-250 from a soil sample collected near Gwangju, Korea which produced macrolide antibiotics. The organism produced 16-membered macrolide antibiotics including chalcomycin (**1**)[4] and a new macrolide antibiotic, 250-144C (**2**). The new antibiotic showed an ultraviolet spectrum which was different from those reported previously for 16 membered macrolide antibiotics. In this paper, we wish to report its structure.

Fig. 1. The structures of 250-144C (**1**), chalcomycin (**2**) and Antibiotic $C_{35}H_{56}O_{13}$ (**3**).



1



2 R=OH
3 R=H

The producing organism was cultured in a tryptic soy broth (tryptone 17 g, soytone 3 g, glucose 5 g, NaCl 2.5 g, K_2HPO_4 2.5 g, distilled water 1 liter, 500 ml) at 28°C on a rotatory shaker (180 rpm) for 2 days, inoculated into a production medium (oat meal 2%, glucose 2%, NaCl 0.4%, yeast extract 0.4%, $CaCO_3$ 0.3%, $MnCl_2 \cdot 4H_2O$ 0.04%, $FeSO_4 \cdot 7H_2O$ 0.04% in distilled water, 12 liters), and cultured for 3 days at 28°C with an air flow of 12 liters per minute and an agitation rate of 200 rpm. The culture broth was extracted with ethyl acetate. The residue of the extract was chromatographed over a column packed with silica gel which was eluted with ethyl acetate-hexane (1:1, 2:1, 3:1) to give 250-144C and chalcomycin. The compound showed UV absorption bands at 215 nm (strong) and 280 nm (weak) which were quite different from the chromophores reported for other macrolide antibiotics (Table 1). The absorption band at 215 nm implied the presence of an α,β-unsaturated lactone, the presence of which was supported by bands at 1716 and 1657 cm^{-1} in the IR spectrum. A weak UV absorption maximum at 280 nm and an IR absorption band at 1720 cm^{-1} supported the presence of an unconjugated ketone group. The presence of two sugars were evident by the doublets at 4.27 (J=7.7 Hz) and 4.56 (J=7.0 Hz) ppm in the ^1H NMR spectrum, and by the signals at 101.1 and 103.6 ppm in the ^{13}C NMR spectrum. The coupling constants (J=7.7 and 7.0 Hz) of the anomeric protons and the chemical shifts of the anomeric carbon atoms suggested that the two sugars are attached to the aglycone by β-glycoside bonds. Hydrolysis of the antibiotic showed two sugar spots on the TLC plate (silica gel, chloroform-methanol, 10:1, visualized with anisaldehyde-sulfuric acid) with identical Rf values of 0.19 and 0.12 as those of the sugars observed from the hydrolyzate of chalcomycin. These observations suggested that 250-144C has chalcose and mycinose at-

Table 1. Physico-chemical properties of 250-144C.

Appearance	Colorless amorphous solid
MP	74~76°C
$[\alpha]_D^{26}$ (c=0.05, EtOH)	−16°
UV λ_{max}^{EtOH} nm	216 ($\varepsilon=3.05\times10^3$), 282 ($\varepsilon$=450)
MW (CI-Mass, $(M+H)^+$, m/z)	670.3920 (Calcd. 670.3927 for $C_{35}H_{58}O_{12}$)
IR ν_{max} cm^{-1} (KBr)	3460, 1716, 1657
Color reaction	(+); Anisaldehyde-sulphuric acid reagent, I_2
	(−); Ninhydrin, Dragendorff reagent
Solubility	Soluble in $CHCl_3$, Et_2O, EtOH, MeOH
	Insoluble in hexane, water
TLC, SiO_2	Hexane-EtOAc (1:4) 0.51
(Rf value)	$CHCl_3$-MeOH (30:1) 0.43

tached to the aglycone part of the molecule. The ^{13}C NMR spectrum revealed signals corresponding to the carbon atoms of the two sugar moieties with similar chemical shifts as those of chalcomycin and other chalcose and mycinose-containing macrolides, neutramycin[5] and antibiotic $C_{35}H_{56}O_{13}$[6]. Their ^{13}C chemical shift-values are compared in Tables 2 and 3. Chemical ionization mass spectrum of compound 250-144C showed product ions at *m/z* 175 and 145 which is consistent with the molecular ions of mycinose and chalcose, respectively through cleavage at the β-glycoside bonds. The coupling constant-values, 7.7 Hz for both $J_{1',2'}$ and $J_{1'',2''}$ of anomeric protons suggested existence of D-forms for the chalcose (D) and the mycinose (E) in Antibiotic 250-144C (Fig. 2).

Most of the ^{13}C NMR signals of the aglycone part of 250-144C were observed at very similar places as those of chalcomycin and antibiotic $C_{35}H_{56}O_{13}$. Their data are compared in Table 4. Analysis of the ^1H-^1H 2D COSY spectrum of **1** revealed five structure units as shown in Fig. 2. Compound 250-144C was confirmed to have the same carbon skeleton as chalcomycin. The presence of an α,β-unsaturated lactone and an unconjugated ketone groups were implied from the UV spectrum. Two signals at 213.7 and 165.6 ppm were assigned to the ketone- and the double-bond conjugated lactone-carbonyl groups, respectively. The double bond

Fig. 2. Spin systems derived from analysis of ^1H-^1H 2D COSY spectrum of 250-144C.

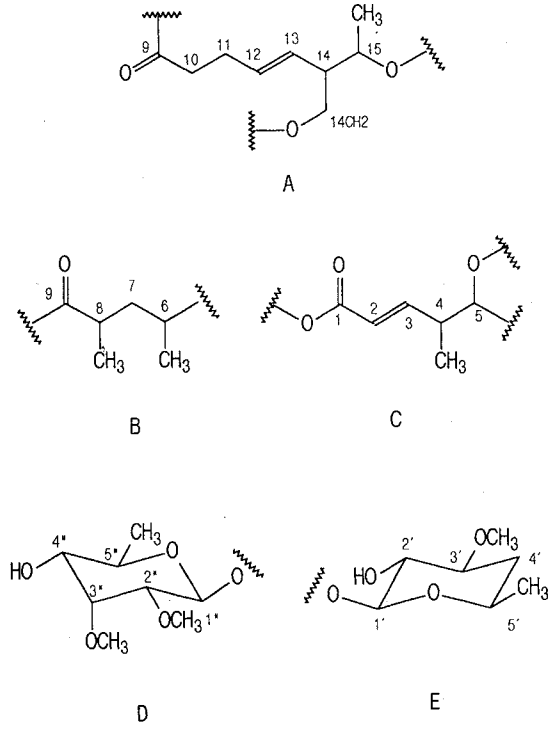


Table 2. Assignments of the ^{13}C NMR signals for mycinose in compound 250-144C and a comparison of the signals assigned to mycinose in chalcomycin, neutramycin, and antibiotic $C_{35}H_{56}O_{13}$.

Position	250-144C	Chalcomycin[a]	Neutramycin[b]	Antibiotic $C_{35}H_{56}O_{13}$[b]
1″	101.1	100.3	100.9	100.9
2″	81.9	81.6	81.5	81.6
3″	79.8	79.4	79.7	79.7
4″	72.7	72.5	72.8	72.7
5″	70.6	70.4	70.7	70.7
5″-CH_3	17.7	17.7	18.1	17.8
2″-OCH_3	59.8	58.5	59.1	59.7
3″-OCH_3	61.7	61.4	61.7	61.7

[a] The chemical shift values of chalcomycin was measured with the compound isolated in our laboratory.
[b] Assignments are from the previously reported refs. 5,6.

Table 3. Assignments of the ^{13}C NMR signals for chalcose in compound 250-144C and a comparison of the signals assigned to chalcose in chalcomycin, neutramycin, and antibiotic $C_{35}H_{56}O_{13}$.

Position	250-144C	Chalcomycin[a]	Neutramycin[b]	Antibiotic $C_{35}H_{56}O_{13}$[b]
1″	103.6	102.9	103.9	103.4
2″	75.1	74.8	74.9	75.1
3″	80.3	80.3	80.3	80.5
4″	36.9	36.7	36.9	36.9
5″	67.8	67.5	68.1	67.8
5′-CH_3	20.8	20.8	20.9	20.9
3′-OCH_3	56.9	56.8	56.8	56.8

[a] The chemical shift values of chalcomycin was measured with the compound isolated in our laboratory.
[b] Assignments are from the previously reported refs. 5,6.

Table 4. ^{13}C NMR chemical shifts of aglycone for 250-144C, chalcomycin and Antibiotic $C_{35}H_{56}O_{13}$.

Position	250-144C[a]	Chalcomycin[a]	Antibiotic $C_{35}H_{56}O_{13}$[b]
C-1	165.6	165.3	165.4
C-2	121.5	120.7	120.6
C-3	150.6	151.5	151.2
C-4	40.8	41.6	41.8
C-5	86.5	87.8	86.9
C-6	34.6	34.0	34.1
C-7	32.9	36.7	32.0
C-8	44.9	79.3	44.7
C-9	213.7	200.1	200.9
C-10	38.5	124.9	125.6
C-11	26.1	146.4	143.9
C-12	132.8	58.7	59.0
C-13	128.7	59.0	58.7
C-14	50.3	49.6	49.5
C-15	69.8	68.7	68.7
14-CH_2	69.9	66.9	76.0
4-CH_3	18.9*	19.1	18.8
6-CH_3	17.2	18.6	17.0
8-CH_3	18.5*	27.8	17.6
15-CH_3	18.9*	18.3	18.4

* Assignments may be reversed.
[a] The chemical shift values of 250-144C and chalcomycin was measured in $CDCl_3$ (125 MHz).
[b] Assignments are from the previously reported ref. 3.

Fig. 3. Correlations obtained from the HMBC NMR spectrum for 250-144C.

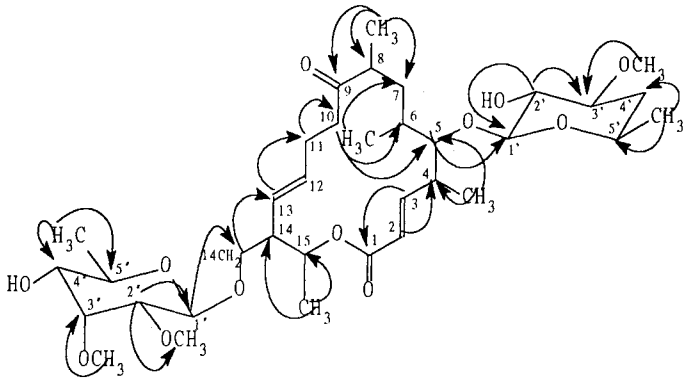


Fig. 4. CI-MS fragmentations of 250-144C.

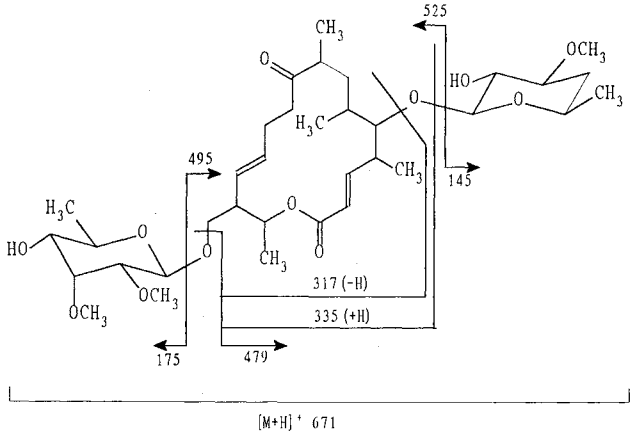


Table 5. ^{1}H NMR chemical shifts of 250-144C ($CDCl_3$, 500 MHz).

Position	Chemical shift (ppm)	Multiplicity	Coupling constant (Hz)
2	5.81	d	15.5
3	6.71	dd	15.5, 10.0
4	2.77	dqd	10.8, 10.0, 6.5
5	3.35	t	10.8
6	1.45	m	3.9, 6.7, 10.8
7	1.40	ddd	5.4, 6.7, 14.7
	1.67	ddd	3.9, 9.7, 14.7
8	2.55	ddd	5.4, 6.9, 9.7
10	2.54	m	
11	2.13	m	6.5
12	5.50	dt	6.5, 15.5
13	5.31	dd	9.0, 15.5
14	2.35	m	9.0
14-CH_2	3.93	dd	4.0, 9.5
	3.43	dd	6.0, 9.5
15	5.07	dq	6.5, 8.5
4-CH_3	1.22	d	6.7
6-CH_3	0.98	d	6.6
8-CH_3	1.09	d	6.9
15-CH_3	1.30	d	6.4
1′	4.27	d	7.3
2′	3.34	dd	7.3, 8.8
3′	3.25	ddd	4.9, 6.4, 8.8
$4'_{eq}$	2.07	ddd	2.0, 4.9, 13.7
$4'_{ax}$	1.14	ddd	4.4, 9.0, 13.7
5′	3.62	d	2.0
6′-CH_3	1.26	d	6.1
2′-OH	2.13	m	
1″	4.56	d	7.7
2″	3.05	dd	7.7, 2.8
3″	3.74	dd	2.8, 2.8
4″	3.19	dd	2.8, 9.0
5″	3.60	dq	5.7, 9.0
2″-OCH_3	3.62	s	
3″-OCH_3	3.53	s	
6″-CH_3	1.25	d	5.7
4″-OH	2.31	m	

is not conjugated with the ketone group since no absorption maximum is observed at 240 nm in the UV spectrum. The four olefinic carbon-signals were observed at 121.6, 150.6, 132.8, and 128.7 ppm. *E*-configurations for the two double bonds were implied from their large coupling values ($J=15.5$ Hz) between their vinylic protons. Compound 250-244C showed a protonated molecular ion at *m/z* 671 in its chemical ionization mass spectrum, and the elemental composition of $C_{35}H_{58}O_{12}$ (obsd. 670.3920; calcd 670.3927) which is one oxygen atom less than that of chalcomycin. The ^{13}C spectrum of chalcomycin showed 6 oxygen atom-bound carbon signals at 58.7, 59.0, 66.0, 68.7, 79.3, and 87.8 ppm, whereas that of 250-144C showed signals at 69.8, 69.9 and 86.5 ppm for 3 oxygen atom-bound carbon atoms, and three other carbon signals around $17\sim50$ ppm. This observation indicated the absence of a hydroxyl and an epoxy groups in the molecule of 250-144C. The assignment of the NMR siganls for the carbon atoms of 250-144C was achieved by ^{1}H-^{13}C correlations established by heteronuclear multiple-quantum coherency (HMQC) experiments. The assignment is compared with that of chalcomycin and Antibiotic $C_{35}H_{56}O_{13}$ in Table 4. The carbon atom at C-8 in **1** which carried no oxygen atom showed a signal at 44.9 ppm that gave almost the same shift (44.7 ppm) as the C-8 atom of antibiotic $C_{35}H_{56}O_{13}$. However, the C-8 carbon atom in chalco-

Table 6. Antibacterial activity of 250-144C.

Test organism	MIC (μg/ml)
Streptococcus pyogenes ATCC 8668	>100
Streptococcus pyogenes C 4003	25.0
Staphylococcus aureus Smith	12.5
Staphylococcus aureus ATCC 29213	12.5
Escherichia coli ATCC 10536	>100
Escherichia coli ATCC 25922	>100
Klebsiella pneumoniae ATCC 10031	>100
Pseudomonas aeruginosa GN 11189	>100
Pseudomonas aeruginosa ATCC 10145	>100
Pseudomonas aeruginosa ATCC 27853	>100
Streptococcus faecalis ATCC 29212	>100
Serratia marcescens ATCC 27117	>100
Salmonella typhimurium C 4045	>100

MICs were determined by the agar dilution method using Mueller-Hinton agar (Difco).

mycin is an oxygenated atom giving a signal at 78.4 ppm. The DEPT spectra revealed five methylene carbons. Two of them at 38.5 and 26.1 ppm were assigned to C-10 and C-11, respectively. The proton at C-15 showed signal at 5.08 ppm as a double quartet ($J=6.5$, and 8.5 Hz). The heteronuclear multiple-bond correlation (HMBC) spectrum revealed ^{1}H-^{13}C long-range couplings of 8-CH_3 with C-7, C-8, and C-9, 6-CH_3 with C-5, C-6, and C-7 (Fig. 3). These couplings further confirmed the partial structures A, B, C of Fig. 2 and the final structure **1**. The positions where sugars were linked were established from the observation of long-range couplings between 5-H and C-1′ and between 1″-H and C-14-CH_2. Thus, attachment of chalcose and mycinose at C-5 and C-14 positions, respectively, in the aglycone was evident. The structure of 250-144C as depicted **1** was supported further by CI-MS analysis as shown in Fig. 4. The fragment ions at m/z 495, 525, and 335 were originated from the loss of sugar units from the molecule[7]. The assignment for ^{1}H and ^{13}C NMR signals for protons and carbons was carried out from these 2-D NMR studies and the results are summarized in Table 5.

These results confirmed that Antibiotic 250-144C is a new chalcomycin type macrolide antibiotic which is a 16-membered lactone with a new chromophore. It had an unconjugated double bond at C-13 with the ketone group. The compound showed weak antibacterial activities (Table 6).

Acknowledgment

The present work was supported by Research Center for New Drug Development and by The Basic Science Research Institute Program, Ministry of Education (BSRI-96-3417).

References

1) TANNER, F. W.; A. R. ENGLISH, T. M. LEES & J. B. ROUTIEN: Some properties of magnamycins, a new antibiotic. Antibiot. Chemother. 2: 441~443, 1952
2) NAKAGAWA, A. & S. OMURA: Part I. Macrolide antibiotics. Chapter 2. Structure and stereochemistry of macrolides. *In* Macrolide Antibiotics. Chemistry, Biology, and Practice. *Ed.*, S. OMURA, pp. 48~64, Academic Press, Inc., New York, 1984
3) BERDY, J.: CRC Handbook of Antibiotic Compounds, Vol. II, Macrocyclic Lactone (Lactam) Antibiotics, pp. 73~77, CRC Press, Inc., Boca Raton, Florida, 1980
4) WOO, P. W. K.; H. W. DION & Q. R. BARTZ: The structure of chalcomycin. J. Am. Chem. Soc. 86: 2726~2727, 1964
5) HAUSKE, J. R.; J. DIBRINO, M. GUADLIANA & G. KOSTEK: Structure elucidation of a new neutralmacrolide antibiotics. J. Org. Chem. 51: 2808~2814, 1986
6) OMURA, S.; A. NAKAGAWA, A. NESZMELYI, S. D. GERO, A.-M. SEPULCHRE, F. PIRIOU & G. LUKACS: Carbon-13 nuclear magnetic resonance spectral analysis of 16-membered macrolide antibiotics. J. Am. Chem. Soc. 97: 4001~4009, 1975
7) JARDIM, M. E.; P. VARENNE & M. A. A. FERREIRA: Structure elucidation of some 14 and 16 membered ring macrolide antibiotics by CIMS. Int. J. Mass Spectrom. Ion Phys. 48: 189~192, 1983